Biomacromolecules

Article

Cite This: Biomacromolecules 2018, 19, 3048−3057

pubs.acs.org/Biomac

Role of Hygroscopic Low Molecular Mass Compounds in Humidity Responsive Adhesion of Spider's Capture Silk

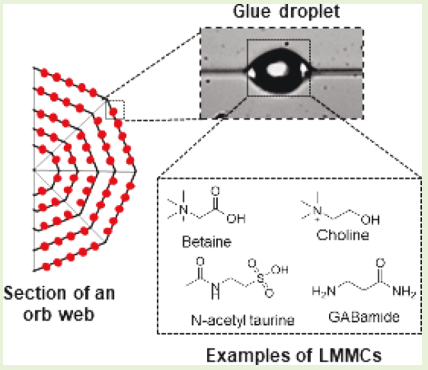
Dharamdeep Jain,[†,‡] Gaurav Amarpuri,[†,‡] Jordan Fitch,[†] Todd. A. Blackledge,[*,§] and Ali Dhinojwala[*,†]

[†]Department of Polymer Science, The University of Akron, Akron, Ohio 44325-3909, United States

[§]Department of Biology, Integrated Bioscience Program, The University of Akron, Akron, Ohio 44325-3908, United States

Supporting Information

ABSTRACT: The aggregate glue in spider webs is composed of hygroscopic low molecular mass compounds (LMMCs), glycoproteins and water. The LMMCs absorb atmospheric water and solvate the glycoproteins to spread and adhere to flying insects upon contact. The glue viscosity varies with humidity and there is an optimum range of viscosity where the adhesion is maximum. LMMCs composition and the humidity at which glue viscosity is optimized vary greatly among spider species. These findings suggest that spiders adapt to forage in diverse habitats by "tuning" LMMCs composition or how LMMCs interact with glycoproteins to control water uptake and adhesion. To test these hypotheses, we analyzed the LMMCs for spiders from diverse habitats and performed water uptake studies on intact glue droplets, isolated glue constituents, and synthetic LMMCs. Even though glue droplets showed differences in water uptake among spider species, we found no differences among species in hygroscopicity of natural or synthetic LMMCs mixtures. This demonstrates that LMMCs composition alone is insufficient to explain interspecific differences in water uptake of spider glues and instead support the hypothesis that an interaction between LMMCs and glycoproteins mediate differences in water uptake and adhesion.



1. INTRODUCTION

Water in bulk or vapor form has always been a roadblock in the performance of synthetic adhesive systems.[1−8] On the other hand, many biological adhesives stick well in the presence of water[9,10] or high humidity.[11−16] Apart from their structural design, the material composition of these natural adhesives contributes to their exceptional performance in the presence of water or humidity. Hence, there is an immense need to understand the design strategy of natural systems to improve adhesion in synthetic systems as well as to fabricate adhesives that can work in the presence of humidity.

While many biological adhesives function at high humidity,[11−16] prey capture adhesives produced by spiders[17−19] is a good system for investigation because they routinely function at fluctuating or high humidity. The sticky capture silk used by araneoid spiders for trapping walking and flying insects, consists of an axial thread of either stretchy flagelliform silk in orb webs or stiffer major ampullate silk in cobwebs coated with microscopic drops of glue from the aggregate silk gland made up of a mixture of organic/inorganic low molecular mass compounds (LMMCs), glycoproteins, and water.[17−39] Glycoproteins make the glue viscoelastic,[33] such that adhesion increases at high peeling rates to trap struggling insects while lasting elasticity retains insects over longer periods of time.[18,33] The humidity response of the capture silk is frequently attributed to the presence of a complex mixture of hygroscopic organic and inorganic LMMCs.[23−25,28,29,31,37−39] In general, LMMCs bear resemblance to biomolecules that serve critical functions in living organisms. These include roles such as (1) osmolytes to maintain internal cellular osmotic pressure to balance the external fluid environment,[40] (2) neuromodulators/neurotransmitters,[31] and (3) stabilizers for protein conformation.[41] In capture silk, LMMCs collect water from the environment and make the glue tacky so that it sticks to natural surfaces including insect cuticles.[42] The LMMCs are present in the water-soluble mass of the glue, which accounts for about 30−60 wt %, of the total mass of the dry web.[38] The water-soluble mass consists ∼60 wt % organic LMMCs composed of mostly polar aliphatic compounds, such as glycine, betaine, choline, putrescine, GABamide, and isethionic acid bearing amine, sulfonate, or acetate functionalities,[23−25,28,29,31,37−39] and ∼10−20 wt % inorganic LMMCs, which include Ca^{2+}, $H_2PO_4^-$, NO_3^-, Na^+, and K^+ ions.[23−25,31,38,39]

There are a number of hypotheses for the function of a complex cocktail of LMMCs in the glue droplets.[23,38] The LMMCs in the glue droplets are hygroscopic and help to retain water in the glue droplets.[25,31] Inorganic/organic LMMCs play a critical role in solvating the glycoproteins.[32] When the LMMCs are washed off, the glue is no longer sticky even after

Received: April 10, 2018
Revised: June 8, 2018
Published: June 13, 2018

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

adding external water to the glue droplet.[32] Other suggested roles include imparting anti-UV and antimicrobial properties, inhibitors for crystallization of the axial flagelliform silk proteins, and acting as toxic agents for prey capture.[23,38] However, only the water absorption and glycoprotein solvation hypotheses have empirical support, so the primary function of the LMMCs is likely increasing adhesion.[32,37] However, it is still not clear why such a wide variety of LMMCs are present in aggregate glue.

Vollrath et al. discovered the LMMCs in the glue droplets and showed that they are hygroscopic.[31] Townley et al.[25] studied the water uptake in different orb-weaving spiders (*Argiope aurantia*, *Argiope trifasciata*, and *Araneus cavaticus*) and demonstrated that the LMMCs are more hygroscopic than the insoluble protein components of the web. But the degree to which LMMCs composition controls variation in glue hygroscopicity and adhesion still remains unexplored. The recent finding that many species of spiders share an optimum viscosity for glue adhesion, but achieve that viscosity at very different atmospheric humidities suggests that LMMCs composition may tune water uptake such that spiders in dry habitat may have LMMCs that absorb water at low relative humidity compared to spiders from wet environments.[11] This leads to two possible, nonmutually exclusive hypotheses for the role of LMMCs in tuning adhesion at very different humidities for diverse glues: (1) Controlling the "hygroscopic strength" of glue droplets. The diverse LMMCs may vary in hygroscopic response across different humidity environments so that when present as mixtures in the glue droplet, their combined hygroscopic activity can tune the overall water uptake of the glue droplet to achieve optimum viscosity at specific RH. (2) Optimizing interactions with glycoproteins: Although glycoproteins of spiders have not been sequenced extensively, chemistry specific interactions between glycoproteins and LMMCs may help to generate humidity responsive adhesion so that water uptake by LMMCs alone will not correlate with variation in glue performance.

In the present study, we explore the link between composition and hygroscopicity of LMMCs with adhesion. We hypothesize that differences in the hygroscopicity of LMMCs composition across species controls variation in adhesion by determining water content, and hence viscosity, of the glue droplets at different humidities. To test this hypothesis, we selected species from significantly different foraging conditions[11,37] to study the hygroscopic properties of their glues and its individual components. First, we describe the chemical composition of organic LMMCs of four species from different habitats. Second, we measure the water uptake of suspended pristine glue droplets (glue in its native form consisting of LMMCs and glycoproteins) in the presence of humidity. Third, we decouple the glue components (LMMCs and glycoproteins) and study the humidity response of "LMMCs" in three different sample types: (a) individual synthetic organic LMMCs found in glue droplets, (b) LMMCs mixtures extracted from capture silk threads, and (c) synthetic LMMCs mixtures mimicking LMMCs compositions in capture silk. Lastly, we test the hygroscopic response of the glycoproteins obtained after washing of the LMMCs from capture silk threads.

2. MATERIALS AND METHODS

2.1. Procurement of Capture Silk Threads. We choose four spider species utilizing different microhabitats: the orb-weavers *Argiope trifasciata* (open fields; Blacksburg, Virginia), *Larinioides cornutus* (near water bodies; Akron, Ohio), and *Tetragnatha laboriosa* (above water; Akron, Ohio)[11] and the cobweb-weaver *Latrodectus hesperus* (widespread across different habitats including semi-arid[37,39,43] purchased from Bugs of America, Arizona). In addition to belonging to different microhabitats, the orb web weavers *Argiope trifasciata*, *Larinioides cornutus*, and *Tetragnatha laboriosa* were selected because each of their glues show maximum adhesion at different humidity conditions (30%, 50%, and 90% RH, respectively).[11] On the other hand, the cobweb weaver *Latrodectus hesperus* was selected as it shows a constant adhesion across a range of humidity conditions (30−90% RH).[37,39] The difference in the adhesive response of each species provides us with the criteria to check our hypothesis of water uptake by organic LMMCs dictating maximum adhesion. *Larinioides cornutus*, *Argiope trifasciata*, and *Latrodectus hesperus* procured from above-mentioned locations were housed in custom built cages in the laboratory to aid web building and subsequent web/thread collection. Freshly spun capture silk from the webs of *Tetragnatha laboriosa* was collected by directly winding whole webs on cardboard frames and glass pipettes from their natural habitat near the Cuyahoga river (Akron, Ohio), since *Tetragnatha* rarely built webs in the laboratory setting.

2.2. Solution-State NMR Measurements. The composition of LMMCs present in the capture silk of the spiders in the study was measured using Solution State NMR. Individual glass pipettes covered with whole orb webs (*Argiope trifasciata* ∼ 10, *Larinioides cornutus* ∼ 60, and *Tetragnatha laboriosa* ∼ 25) were collected. In the case of cobweb weaver *Latrodectus hesperus*, the sticky capture glue present in lower part of the web as individual vertical strands known as gumfoot silk were collected. About 750 of gumfoot strands were collected for analysis.[37] The collected capture silk from each of the species was washed with deionized water for 10 min followed by lyophilization of water washings to get LMMCs (Figure S1). A part of the extracted LMMCs mixtures for each spider silk was dissolved in 99.96% deuterated water (1 mL) (Cambridge Isotope Laboratories) and filled in the 5 mm NMR tube (Norell) for chemical characterization. All ^{1}H experiments were carried at 298 K on Varian Mercury 300 MHz spectrometer. For quantification, proton spin−lattice relaxation experiments were performed and the longest relaxation time was about 4 s for each of the extract sample. So, accordingly, ^{1}H experiments were conducted by setting the recycle delay to $5 \times T_1 \sim 20$ s, respectively. The ^{1}H experiments were conducted with scan size ∼512, acquisition time ∼2.9 s, and pw90−15−22 μs. The peaks were analyzed and integrated with ACD/NMR software to calculate the relative composition of each LMMC in the glue.

2.3. Hygroscopicity of Suspended Pristine Glue Threads. The hygroscopic water uptake of the suspended pristine capture glue threads was measured by monitoring the change in volume using a Olympus BX53 microscope with 20× and 50× objectives and a Photron FASTCAM SA3 camera at different relative humidities.[11] A custom-built humidity chamber controlled the ambient humidity around the capture silk mounted on a glass fork. The same glue droplet was observed as the humidity was increased from 10%, 30%, 60% to 90% RH. At each humidity, the droplet was observed to equilibrate quickly but pictures were taken after 5 min for consistency. The droplet volume was calculated using the formula defined by Liao et al.[44] The change in volume of the glue droplet is assumed to be only because of the water uptake and hence, the increase in glue droplet volume is measured at different humidities. Please note that the volume measurement from imaging is the actual glue volume increase, which includes the effect of glue compositions, size and droplet curvature. A sample of 12−20 glue droplets from 3−5 spiders were tested for each spider species. A two-sample *t* test was used to compare the changes in volume.

2.4. Hygroscopicity of Synthetic Organic LMMCs, Natural LMMCs Extracts, Synthetic LMMCs Mixtures, Washed Glue Threads, and Pristine Immobilized Glue Threads. To understand the hygroscopic nature of different components of glue, we measured the water uptake of a variety of samples that included the following: (a) Synthetic organic LMMCs (Figure 1a), in order to

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

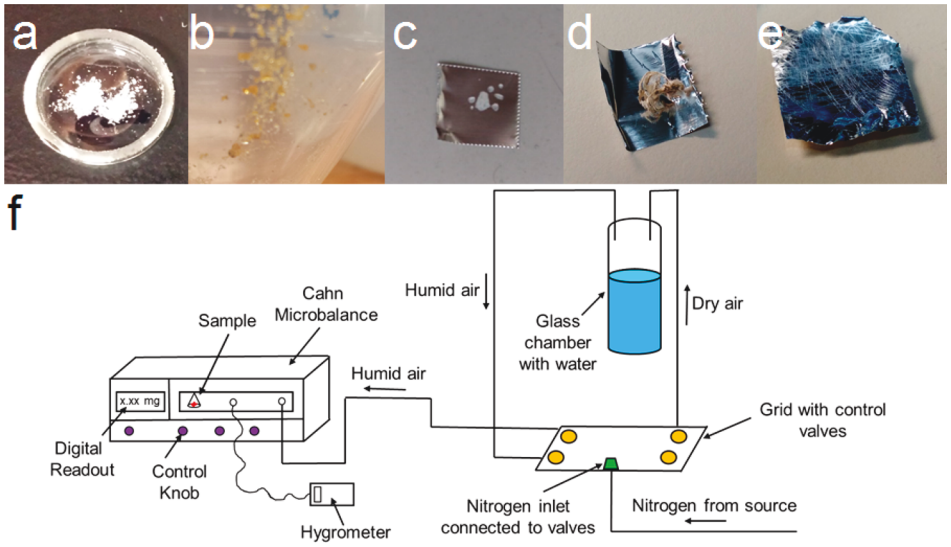


Figure 1. Water uptake measurements. (a–e) Different types of samples for water uptake measurements: (a) synthetic organic LMMC (L-proline shown here), (b) extracted LMMCs in a centrifuge tube after lyophillization, (c) synthetic mixture mimicking LMMCs recipe from glue of *Tetragnatha laboriosa*, (d) washed capture silk, and (e) pristine immobilized glue threads on an aluminum foil. All natural samples (b, d, and e) shown are related to the capture silk from the webs of *Larinioides*. (f) Set up for water uptake experiments showing different parts, including the custom-built humidity controller and Cahn Microbalance.

establish the individual hygroscopic response of various LMMCs present across glues of diverse species of spiders; (b) Natural extracted LMMCs mixtures from whole webs (Figures 1b and S1), to understand the hygroscopic nature of the cocktail of LMMCs based on the composition they are found in the capture silk; (c) Synthetic LMMC mixtures (Figure 1c) prepared based on the composition found in glue, to compare the activity with natural extracts; (d) Washed capture silk (Figure 1d) to study the trend in water uptake of capture silk threads in the presence of only glycoproteins; and (e) Pristine immobilized glue threads (Figure 1e), to compare with washed capture silk and separate the hygroscopic response of LMMCs from glycoproteins.

Method of Measurement. The hygroscopicity of the five different types of samples described above was established by studying the water uptake using a Cahn microbalance attached to a custom-built humidity set up (Figure 1f). The microbalance was fitted with an acrylic sheet that served as inlets for hygrometer (VWR) and humidified/dry air. The sample placed on preweighed aluminum foil was loaded on the suspended pan in the microbalance. To confirm there was negligible uptake by aluminum foil, control experiments were performed with empty foil throughout the range of conditions (30%, 60%, and 90% RH). The sample was dried at 10% RH until a constant reading displayed on the readout. This mass of the aluminum foil was subtracted from the mass of the dried sample to obtain the mass of the sample. Next, the humidity was increased to 30%, then 60%, and finally 90% RH, and at each humidity after the desired environment was equilibrated, readings were taken at every 5 min for a total of 20 min. In case of individual synthetic LMMCs, the sample was kept in an oven at 50–60 °C for 30 min to expel water and then immediately transferred on the pan in microbalance and dried again at 10% RH, followed by steps discussed previously. The final reading at each humidity (30%, 60%, and 90% RH) was taken for calculating the % water uptake in each case. The water uptake was normalized to the weight measured at 10% RH condition. A set of three measurements were done for each type of sample. The statistical analysis was carried out using ANOVA.

Sample Preparation. a. Synthetic Organic LMMCs. The LMMCs included N-acetyl putrescine, betaine, GABA, isethionic acid, choline chloride, taurine, putrescine, L-proline, β-alanine (all from Sigma-Aldrich), N-acetyl taurine (synthesized in laboratory),[45] GABamide (provided by Dr. M.A. Townley, University of New Hampshire), and glycine (Calbiochem). About 1–2 mg of LMMC was taken on a preweighed aluminum foil and analyzed for the uptake.

b. Natural LMMCs Extract from Webs. Whole webs collected on glass pipettes from *Larinioides cornutus* (~20 webs), *Argiope trifasciata* (two sets of samples with 25 and 17 webs each, respectively), *Tetragnatha laboriosa* (~20 webs), and *Latrodectus hesperus* (two sets of samples with 725 and 380 gumfoot strands each, respectively) were washed as per procedure described in the solution state NMR section. The LMMCs extract was then placed on previously weighed strip of aluminum foil to initiate the measurements.

c. Synthetic LMMCs Mixtures. Two set of synthetic mixtures based on the LMMCs compositions of *Argiope trifasciata* and *Tetragnatha laboriosa* were prepared by mixing the individual synthetic organic LMMCs described above. Both these spiders were selected because their adhesion shows maximum at drastically opposite humidity values) *Argiope* ~ 30% RH and *Tetragnatha* ~ 90% RH). Therefore, these systems become ideal choices to test whether LMMCs compositions dictate adhesion differences. The recipe of each mix was based on the composition found by NMR analysis in the present study (see Results and Table S1). About 10 mg of mix was prepared by weighing the respective LMMCs and placing them in a glass petridish. The petridish was then placed overnight in a small humidity chamber to initiate homogeneous mixing of the compounds. After a clear liquid pool was formed, a drop of mixture (~1–2 mg) was taken with a micropipette and placed on a preweighed aluminum foil, followed by conducting the water uptake measurements described previously.

d. Washed Capture Silk. Two to three webs of *Larinioides cornutus* and *Argiope trifasciata* were collected on glass pipettes and given repeated washes in deionized water to remove the water-soluble compounds. The pipet with the washed silk on it was then allowed to dry overnight in air. Later, the dried silk was scrapped from the pipet and used for measurements.

e. Pristine Immobilized Capture Silk. Freshly spun pristine sticky silk threads were collected on a strip of aluminum foil directly from the webs of *Argiope trifasciata* and *Larinioides cornutus* and used for measurements. The silk, from the two species, *Larinioides cornutus* and *Argiope trifasciata* was used for analysis (comparison between washed and pristine) due to ease of sample procurement and preparation. All biological samples were stored in refrigerator and synthetic LMMCs in desiccator until measurements were done.

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048–3057

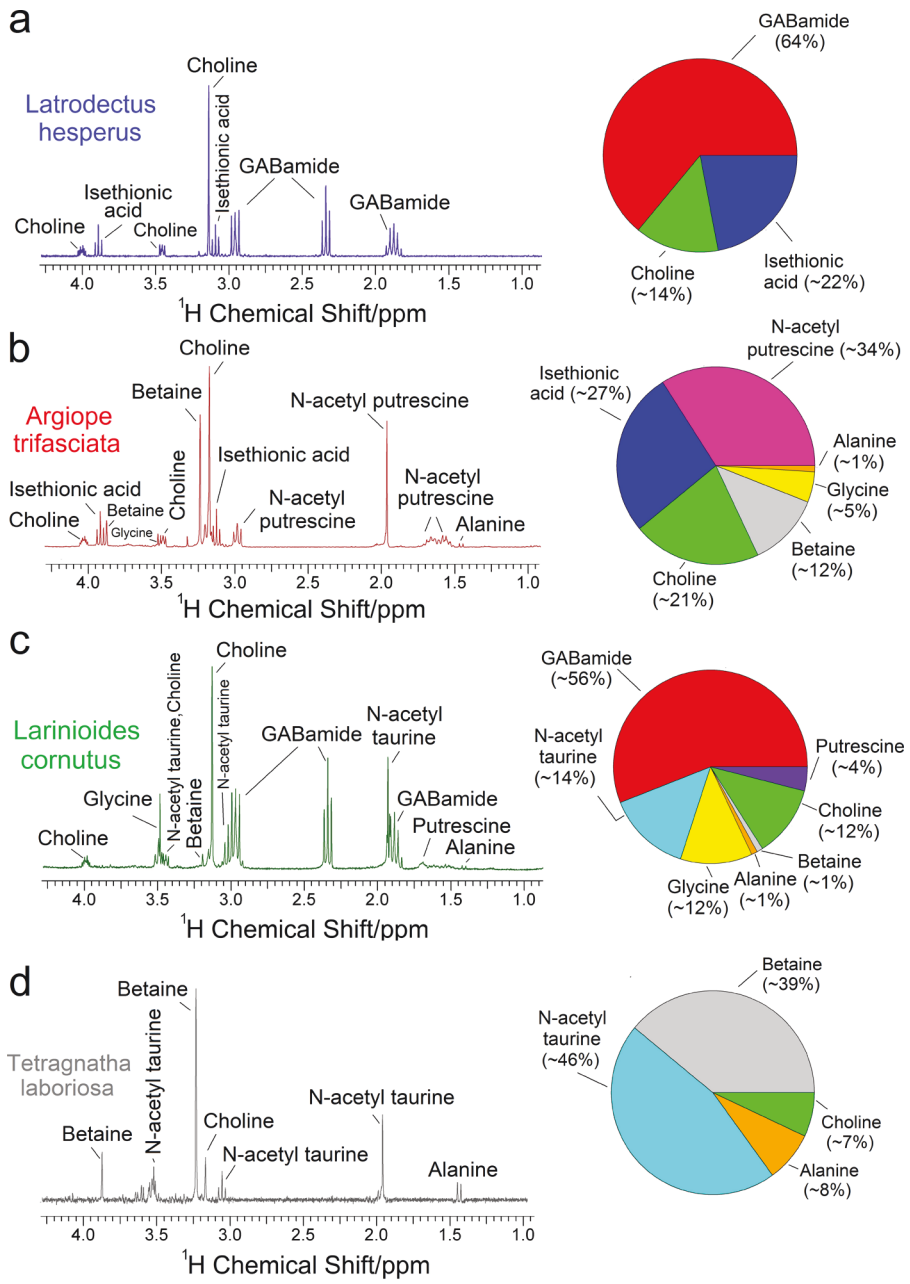


Figure 2. Diversity of organic LMMCs in capture silk. (a–d) ^{1}H solution-state NMR spectra of the extracted LMMCs mixtures from the webs of *Latrodectus hesperus*, *Argiope trifasciata*, *Larinioides cornutus*, and *Tetragnatha laboriosa*, respectively. Each spectrum is accompanied by a color coded pie chart (each color representing a distinct LMMCs compound) showing the details of relative composition of each LMMCs component in different glues.

3. RESULTS

3.1. Solution-State NMR of LMMCs Extracts from Webs. The water-soluble LMMCs extracts (Figure S1) from the capture silk threads of *Latrodectus hesperus*, *Argiope trifasciata*, *Larinioides cornutus*, and *Tetragnatha laboriosa* were analyzed for organic LMMCs compositions by ^{1}H solution-state NMR. The spiders selected optimize their glue adhesion at very different RH and clearly the glue from each species is a combination of distinct organic LMMCs combinations ranging from three in *Latrodectus hesperus*, five in *Tetragnatha laboriosa*, six in *Argiope trifasciata* to seven in *Larinioides cornutus* (Figure 2 and Table S1). Across species, LMMCs differ not only in the chemical properties but also in their composition. One or two LMMCs dominated the composition of each species, but LMMC identity differed among species (*Latrodectus hesperus*: GABamide ∼64%; *Argiope trifasciata*: N-acetyl putrescine ∼34% and isethionic acid ∼27%; *Larinioides cornutus*: GABamide ∼56%; *Tetragnatha laboriosa*: N-acetyl taurine ∼46% and betaine ∼39%). Hence, there is tremendous diversity of the LMMCs present in the glue of spiders.

3.2. Water Uptake of Suspended Pristine Silk Threads. After establishing the differences in organic LMMCs compositions among the capture silks of species, we started with the series of water uptake studies. The capture silk in its native state is in suspended form where water uptake is a result of a combination of factors: hygroscopic material, droplet shape, exposed surface area of glue droplets.

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

Immobilizing the glue droplets on the substrate may change the rate and extent of water uptake.[46] Hence, we used microscopy of suspended glue droplet to measure the water uptake by glue droplet. Figure 3a–d shows a single glue droplet of *Latrodectus hesperus* under increasing humidity. Notice that the glue droplet size increases significantly with an increase in humidity. We calculated the glue volume using the formula described by Liao et al.[44] and plotted the increase in volume of pristine glue at each humidity with respect to volume at 10% RH, in suspended state for the four spider species (Figure 3e). Clearly, glue of *Tetragnatha laboriosa* absorbs significantly less moisture at 90% RH than the other three species (Figure 3e and Table S2). This observation supports the spreading and viscosity observation where the *Tetragnatha laboriosa* glue shows higher viscosity than the species from drier habitats tested at 90% RH.[11] The normalized increase in volume of the other three species is similar (Table S2) but the glue of some species, specially *Argiope trifasciata*, shows maximum adhesion around 30–50% RH[11] and therefore probably had water present at 10% RH. ATR-IR measurement of *Argiope* glue show bound water even at 0–10% RH (Figure S2). *Latrodectus hesperus* glue is unique because its adhesion is constant over 30–90% RH[37] even though the glue absorbs moisture and shows a ~1000× drop in viscosity with an increase in humidity. This is unlike the other orbweb spider species, where adhesion changes with a change in glue viscosity. Hence, we see a difference in the water uptake of native glue threads among species of different habitats. Next, based on the results of differences in LMMCs compositions and water uptake of glue threads among species, we tested whether the diverse LMMCs composition (Figure 2) modulates the water uptake of glue that matches the adhesion performance.

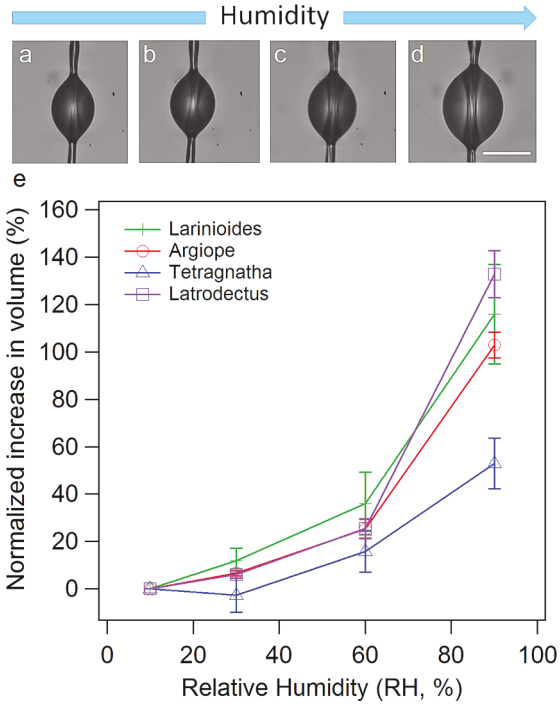


Figure 3. Effect of humidity on the volume of suspended capture silk droplets. (a–d) Optical images of the single capture glue droplet of *Latrodectus* exposed to 10%, 30%, 60%, and 90% RH, respectively. Scale bar is 50 μm. (e) Normalized increase in volume (%) of pristine capture silk glue belonging to *Latrodectus*, *Argiope*, *Larinioides*, and *Tetragnatha*, as a function of relative humidity. The water uptake increases with humidity is similar in *Larinioides*, *Argiope*, and *Latrodectus*, while *Tetragnatha* was observed to absorb much less water with humidity at 90% RH. The error bars show ±95% confidence interval and sample size is ≥15.

3.3. Hygroscopicity of LMMCs. Water uptake studies for LMMCs were performed in three different ways (Figure 1a–c,e). First, to establish the individual hygroscopic response of the various LMMCs present in glue, water uptake by 12 synthetic organic LMMCs was measured. Past attempts in this direction were limited to specific LMMCs and only took in account humidity range until 60% RH.[25,31] Here, we present an extensive analysis of the hygroscopic response of organic LMMCs from 30% to 90% RH. Figure S3 and Table S3 show the normalized water uptake of various organic LMMCs found in capture silk. The water uptake was normalized to the weight of the sample at 10% RH. The control sample (aluminum foil) did not show a significant increase in water uptake upon increase in environment humidity. The common organic LMMCs detected in the capture silks across species (Figure 2) varied in hygroscopic response and were broadly classified as low, moderate, and high (Figure 4) on the basis of their total water uptake capacity at 90% RH. Overall, the water uptake by LMMCs increases with increase in the humidity, with total water uptake ranging widely 6–120% at 90% RH for different organic LMMCs. Low hygroscopic activity LMMCs including glycine, taurine, and alanine are described as LMMCs, which overall show inertness to water absorption with low water absorption (<20% water uptake by mass with respect to mass at 10% RH) at higher humidity (90% RH). Moderately active LMMCs include putrescine, choline chloride, and GABA and absorb in the range of 40–70% by mass at 90% RH. Highly active LMMCs include *n*-acetyl putrescine, *n*-acetyl taurine, betaine, GABamide, isethionic acid, and L-proline that absorb between 80 and 120% by mass (wrt mass at 10% RH) at 90% RH. Hence, we find a diversity in hygroscopicity of the organic LMMCs present in glue. Upon exposure to 90% RH, less active LMMCs maintain their powder form and do not turn into a liquid pool as seen in case of all of the moderately and highly active LMMCs. Also, among the LMMCs only choline chloride and putrescine turn into liquid pools as soon as they

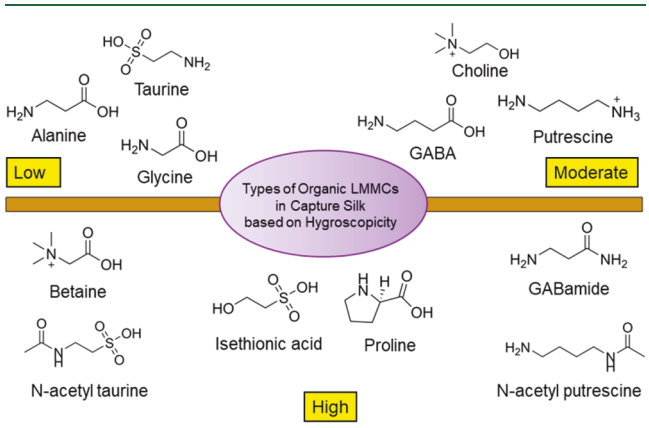


Figure 4. Classification of organic LMMCs on the basis of hygroscopicity. Schematic illustrating the major organic LMMCs in the capture silk across various species. The LMMCs are divided into less active, moderately active and highly active based on the total water uptake of synthetic LMMCs at 90% RH.

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048–3057

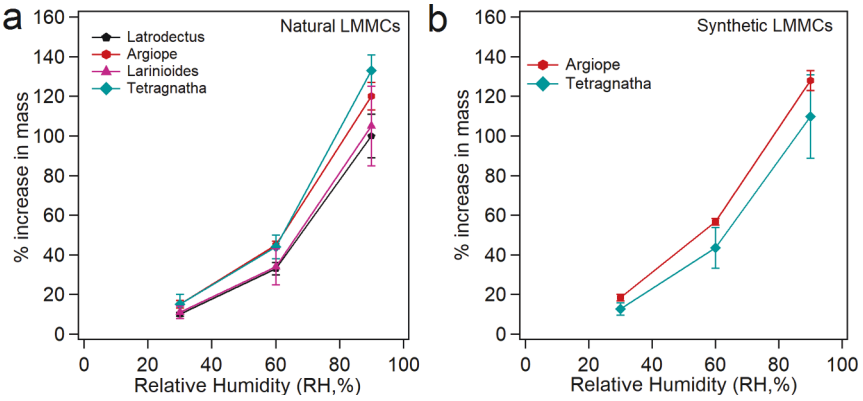


Figure 5. Water uptake of natural LMMCs extracts and synthetic LMMCs mixtures. Figure 5a shows normalized increase in mass (%) of natural LMMCs extracts obtained from webs of *Latrodectus*, *Argiope*, *Larinioides*, and *Tetragnatha* as a function of humidity. Notice that overall the mixture of LMMCs absorb more water with an increase in humidity across species. Figure 5b shows the comparison of normalized increase in mass (%) for synthetic LMMCs mixtures species from extreme habitat: *Argiope* and *Tetragnatha*. We do not observe a significant difference in water uptake of mixtures between species at a particular humidity as observed in suspended glue droplets (for statistics, see Table S4). These observations support glycoprotein−LMMCs interaction hypothesis that regulates the water uptake in glue.

are exposed to the external environment (observed at 20−30% RH).

In the second set of experiments, we probed the hygroscopic strength of natural LMMCs mixtures extracted from capture silk. The extracted LMMC will have both organic and inorganic water-soluble components of pristine glue. Figure 5a shows the water uptake by natural LMMCs extracts of the four spider species, *Latrodectus hesperus*, *Argiope trifasciata*, *Larinioides cornutus*, and *Tetragnatha laboriosa*. The mixtures present in glue are hygroscopic and increase in water uptake with increase in humidity. However, at each humidity studied, we do not see any significant differences among different species in the water uptake behavior of natural LMMCs mixtures, unlike the trend observed in pristine silks' water uptake (Figure 3; see statistical analysis, Figures S4−S6). As an example, (1) *Argiope trifasciata* is active at low humidity and has maximum adhesion at 30% RH.[11] But its extracted LMMCs do not show any difference in hygroscopic water uptake at 30% RH as compared to other species, specifically *Tetragnatha*, which has lower adhesion at 30% RH. (2) Unlike suspended glue droplets, we did not observe a reduced water absorption for *Tetragnatha* LMMCs at 90% RH. (3) *Latrodectus hesperus* LMMCs extract does not show a consistent uptake behavior similar in case of its adhesion over different relative humidity conditions.[37]

However, it is important to point out that the trend in the normalized water uptake is challenging to interpret as we are normalizing with respect to the dry weight at 10% RH. Spectroscopic data (Figure S2) suggests that water is present at low humidity in the glue and that different amount of water concentration could be present in the glue from different species at 10% RH. Based on the composition found by NMR analysis and hygroscopic performance of synthetic LMMCs, we calculated the theoretical mass uptake for each LMMCs mixture recipe (Figure S7). The mass was calculated by adding the weighted average of water uptake of individual LMMCs (eq 1, Table S3) present in the species. The weights were determined by the proportion of LMMCs composition detected using solution state NMR (Table S1). Theoretical water uptake at *X*%. RH is calculated using the following equation:

$$\text{Total Uptake} = \sum_{n=1}^{n} w \times \text{water uptake of } n \text{ at } X\% \text{ RH} \quad (1)$$

where w = % of LMMC from solution state NMR and n = different LMMCs. We found no significant differences among the hygroscopic performance of mixtures across species in a set of humidity conditions.

In the last set of LMMCs experiments, we formulated synthetic LMMCs mixtures similar to the compositions of *Argiope* and *Tetragnatha* in order to double check the observed trend in the water uptake of natural extracts. As stated earlier, *Argiope* shows maximum adhesion at 30% RH, while *Tetragnatha* shows at 90% RH. Clearly no distinction in the water uptake properties for synthetic LMMCs mixtures is seen between the two species across the humidity conditions (Figure 5b and Table S4). The trend is similar as seen in the natural extracts of the glue belonging to the species of spiders (Figure 5a). Results pertaining to natural and synthetic mixtures suggest that the water uptake by LMMCs alone does not control the glue viscoelasticity and ultimately adhesion. Thus, we further probed the water uptake by the glycoproteins.

3.4. Hygroscopicity of Glycoproteins. To understand the role of glycoproteins in mediating viscoelasticity, we studied the water uptake of washed capture silk threads and compared it with the behavior of pristine silk threads in the presence of humidity. Washing silk with water removes the LMMCs and leaves the residual glycoproteins.[32,37] In absence of LMMCs, glycoproteins lose adhesion as seen previously in our macro and molecular level studies.[32,37,39] Figure 6 depicts the comparison of the hygroscopic behavior of pristine immobilized silk threads (Figure 2e) with washed silk threads (Figure 2d) of *Argiope trifasciata* and *Larinioides cornutus*. It is evident that the water uptake is drastically reduced (<20% at 90% RH) for both the species and glue with glycoproteins alone does not take up water as much as in the presence of LMMCs (pristine sample). The behavior of washed glue in the presence of humidity relates to the loss in adhesion and reiterates the synergistic play of both LMMCs and glycoproteins in preserving adhesion of capture silk.

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

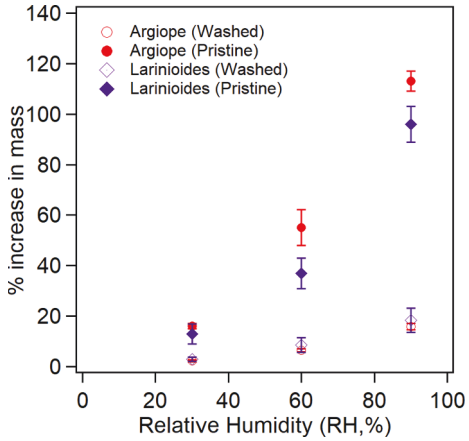


Figure 6. Water uptake of glycoproteins. Normalized increase in mass (%) of washed glue (residual proteins) vs pristine glue for *Argiope* and *Larinioides* as a function of relative humidity. The pristine silk which includes both proteins and LMMCs absorbs significantly more water across humidities, as compared to washed silk which contains residual proteins.

4. DISCUSSION

The adhesion of capture silk threads in spider webs depends on relative humidity and the adhesion is maximum when the glue absorbs enough moisture to optimize both the interfacial adhesion and cohesive strength of the glue.[11] The humidity response of capture silk is often linked to the presence of a cocktail of organic and inorganic hygroscopic LMMCs that are a part of the water soluble mass of the capture silk. The humidity at which viscosity is optimized varies greatly across species[11] and this suggest that the differences in the LMMCs in capture silk of spiders from different habitat explains the differences in the humidity response of the adhesion forces. We found diversity in organic LMMCs (Figure 2 and Table S1) across species spanning different habitats. LMMCs composition varies among many other spider species in the literature[25,31] and that diversity coupled with the hygroscopic response of synthetic LMMCs (Figures 4 and S3 and Table S3) suggest at possible inter species differences in the hygroscopicity of glues. For instance, glues of *Argiope* and *Larinioides* are made up of ∼70−75% "high" hygroscopic compounds, while *Tetragnatha* glue is ∼85−90% "high" hygroscopic compounds (majorly two highly hygroscopic LMMCs, betaine, and N-acetyl taurine, see Table S3).

The direct measurements of the hygroscopicity of the aggregate glue (comprising of both LMMCs and glycoproteins) of *Argiope* and *Tetragnatha* shows clear differences in water uptake at 90% RH (Figure 3e). *Tetragnatha* glue volume increased only ∼40% as compared to ∼100−140% increases for the other three species (*Argiope trifasciata*, *Larinioides cornutus*, and *Latrodectus hesperus* (Figure 3e and Table S2). This correlates well with the viscosity response of *Tetragnatha*'s glue, which maintains a higher viscosity until 90% RH, the humidity where it reaches a maximum adhesion.[11]

However, on comparing the quantity of water uptake for both natural and synthetic mixtures, the results are very similar for all four species investigated here. The water-soluble extracts from all four species showed water uptake of ∼10% for 30% RH, 35−40% for 60% RH, and 100−130% at 90% RH. No particular trend is observed specially in the case of *Tetragnatha* LMMCs, whose uptake at 90% RH looks similar to the activity of LMMCs extracts of other three species. Previous work on hygroscopicity of water-soluble LMMCs extracts based on *Argiope aurantia* and a comparison between *Argiope aurantia*, *Argiope trifasciata*, and *Argiope cavaticus* have also reported the water uptake numbers in the similar range over humidity ranging from 20 to 60% RH.[25,31] Moreover, the work by Townley et al.[25] reported the water-soluble extract of the three species differed in the LMMCs compositions, but it was difficult to distinguish the differences in hygroscopicity of the webs. Measuring the water uptake of synthetic LMMC mixtures (Figure 5b) for the two dramatically opposite (in terms of adhesion and viscosity) performing species, *Argiope* and *Tetragnatha*, we find that the increase in mass of synthetic LMMCs with humidity was similar in both the species. This was the same trend we observed using the natural LMMCs extracts. We have also calculated the theoretical water uptake based on the hygroscopicity of the individual organic LMMCs (Figure S7). Interestingly, these theoretical predictions match the water uptake we measured for the synthetic mixtures, which is again consistent with our conclusion that the differences in the hygroscopicity of the LMMCs does not explain the humidity response of the adhesive glue of *Argiope* and *Tetragnatha*. In addition to the hygroscopicity of the LMMCs, it is also possible that the amount of LMMCs could be different in *Tetragnatha* and other three species. We calculated that *Tetragnatha* glue is composed of ∼50% by weight in comparison to the weight of the whole web. We observed the same range of composition for the other three species (*Latrodectus* ∼ 57%, *Argiope* ∼ 43%, and *Larinioides* ∼ 40%). Thus, neither the hygroscopicity of the LMMCs nor the amount of LMMCs present in the web explain the humidity response of these four species of spiders. It is clear that the LMMCs in combination with the glycoproteins show much more striking differences in water uptake than the LMMCs by themselves, which supports the second hypothesis that it is an interactions between LMMCs compounds and the glycoproteins, which determines glue hygroscopicity and ultimately the variation among spider species in adhesion.

There is evidence of molecular interaction between the LMMCs and glycoproteins based on adhesion and solid-state NMR measurements.[32,39] For example, glycoproteins present in glue are sticky in the presence of LMMCs.[32,37,39] If the LMMCs are washed away, glycoproteins became rigid and fail to take in water (less than 20% at 90% RH; Figure 6). The solid-state NMR results show differences in mobility of glycoproteins in the presence and absence of LMMCs.[32,39] Apart from this evidence, there are two major factors that provide support to the interaction hypothesis. (a) Variation in glycoprotein chemistry: Glycoproteins present in the capture silk are composed of two proteins ASG1 and ASG2 deduced based on cDNA studies.[36] Recently, a ∼10% variation has been observed in the sequence of proteins sequence from three species.[47] More evidence for glycoprotein diversity includes the optical images of the capture threads published by Opell et al.[48] for 17 species orb web weavers indicating the differences in glycoprotein granule morphology (appearance, shape, length, width, area and volume). Also, when comparing capture thread glue produced by orb web weavers (viscid threads) versus cobweb weavers (gumfoot threads), we found differences in the morphology of the glycoproteins with viscid glue assuming core shape structure and gumfoot glue being fluid-like and spreading over the underlying fiber.[34,37] Isolation of glycoproteins from capture threads is tedious as compared

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

to the LMMCs as the glue proteins tend to stick to the underlying axial thread. Devising strategies to remove glycoproteins from capture threads to better characterize their material properties should form the basis of future study. Nevertheless, our observations provide clues that variation in glycoproteins is important in interacting with diverse LMMCs functionalities in glue and ultimately tuning the adhesion response among species of spiders. (b) Glue viscoelasticity: During peeling of glue, energy is spent in breaking/deforming both interfacial bonds and bulk polymer network.[49] For viscoelastic adhesives, such as spider glue, the energy spent in the bulk can be significantly higher than the energy spent in breaking interfacial bonds. The viscoelasticity of the glue determines the energy spent in the bulk during peeling. We believe that spider glue viscoelasticity is constant and optimized at the maximum adhesion conditions across spider species. The glue viscoelasticity is dependent on multiple variables, including the LMMCs−glycoprotein interactions and also LMMCs/glycoprotein concentrations. Direct measurement of LMMCs and glycoprotein concentration for glue droplet for each species is challenging due to limitations in glue sample collection. However, we used a combination of gravimetric and optical measurements to infer the protein concentration (Supporting Information, Text S1). The calculated protein concentrations vary widely with humidity and are not similar at the maximum adhesion condition for the four species. This finding again supports the hypothesis that these diverse LMMCs are not just for water uptake but also for specific interactions with glycoproteins to modulate viscosity and ultimately adhesion.

Protein−LMMCs interactions form an important part of various biological mechanisms. Proteins in the presence of LMMCs, osmolytes or compatible solutes have stabilized conformational structures.[40] LMMCs also mediate the "salting in" and "salting out" mechanisms by interacting with proteins, leading to precipitation or crystallization.[41] It is likely that the organic LMMCs are acting as ionic liquids for the solubilization and stability of glycoproteins. Importantly, ionic liquids based on choline have been extensively used for protein dissolution. Inorganic LMMCs interact with adhesive proteins produced by marine organisms such as oysters,[50] as well as in glycoprotein function in synovial fluids.[51] Synthetic systems such as hydrogels,[52] electrospun fibers,[53] polymer brushes,[54] membranes,[55] and more recently adhesive joints[56] have also been shown to alter function on the basis of interaction of LMMCs with macromolecular structures such as polymers. These studies further support the hypothesis that LMMCs in the glue interact directly with glycoproteins and the water uptake can be modulated by this interactions and the differences in water uptake between *Tetragnatha* and other three species could reflect the differences in the chemical composition of glycoproteins instead.[47] This study suggests a need to map out the protein sequences of glycoproteins for species of spiders from different habitat.

5. CONCLUSION

The present study aimed to better establish the role of LMMCs in capture silk adhesion. We hypothesized that the composition of LMMCs present in the glue droplet tune their water uptake capacity to optimize the viscosity and maximum adhesion. We found that the glues of *Latrodectus hesperus*, *Argiope trifasciata*, *Larinioides cornutus*, and *Tetragnatha laboriosa*, each of which forages in different microhabitats, vary widely in their chemical composition of organic LMMCs. The water uptake of pristine suspended glue droplets indicated differences in water uptake with humidity, with *Tetragnatha* glue taking up less water at 90% RH as compared to other species, matching its improved adhesion performance. The contribution of LMMCs was assessed by quantifying the hygroscopic performance of synthetic organic LMMCs, natural LMMCs mixtures, and synthetic LMMCs mixtures. The synthetic organic LMMCs were found to be hygroscopic and were classified as low, moderately, and highly active. On their own, the water uptake behavior of LMMCs mixtures, both natural and synthetic were found to be inadequate to explain the humidity responsive adhesion. Finally, glycoproteins in absence of LMMCs showed a reduced water uptake activity. These results reiterate the role of LMMCs in interacting with glycoproteins to mediate water uptake and capture silk adhesion. Understanding these interactions of individual LMMCs moieties with glycoproteins may add to the current knowledge of role of diverse LMMCs in capture silk, the natural design of capture silks, silk adhesion mechanism, and fabrication of similar synthetic mimics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.8b00602.

Additional data, including figures of LMMCs extracts, ATR-IR data of *Argiope trifasciata* glue at 10% RH, water uptake of synthetic LMMCs, ANOVA statistical analysis of natural LMMCs extracts at 30%, 60%, and 90% RH, and theoretical water uptake by synthetic LMMCs mixtures; tables showing LMMCs composition across species, statistical analysis of water uptake by suspended glue droplets, water uptake by synthetic LMMCs, and statistical analysis of synthetic LMMCs mixtures; text related to glycoprotein concentration in glue of different species (PDF).

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: ali4@uakron.edu.
*E-mail: tab27@uakron.edu.

ORCID

Dharamdeep Jain: 0000-0002-2253-5714
Gaurav Amarpuri: 0000-0002-2655-5974
Ali Dhinojwala: 0000-0002-3935-7467

Author Contributions

‡These authors contributed equally to the project.

Funding

The project has been funded by IOS-1257719, NSF-DMR 1610483, and NSF-1659531 (NSF-REU program).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Dr. Mark A. Townley (University of New Hampshire) for providing samples of GABamide and N-acetyl putrescine, Kaushik Mishra for synthesizing N-acetyl taurine, and Edward Laughlin for the fabrication of humidity controller.

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

REFERENCES

(1) White, C.; Tan, K. T.; Hunston, D.; Steffens, K.; Stanley, D. L.; Satija, S. K.; Akgun, B.; Vogt, B. D. Mechanisms of criticality in environmental adhesion loss. *Soft Matter* **2015**, *11*, 3994−4001.

(2) Abdel Wahab, M. M. Fatigue in Adhesively Bonded Joints: A Review. *ISRN Mater. Sci.* **2012**, *2012*, 1−25.

(3) Brewis, D. M.; Comyn, J.; Raval, A. K.; Kinloch, A. J. The effect of humidity on the durability of aluminium-epoxide joints. *Int. J. Adhes. Adhes.* **1990**, *10*, 247−253.

(4) Cognard, J. Influence of water on the cleavage of adhesive joints. *Int. J. Adhes. Adhes.* **1988**, *8*, 93−99.

(5) Kinloch, A. J.; Little, M. S. G.; Watts, J. F. The role of the interface in the environmental failure of adhesive joints. *Acta Mater.* **2000**, *48*, 4543−4553.

(6) Korta, J.; Mlyniec, A.; Uhl, T. Experimental and numerical study on the effect of humidity-temperature cycling on structural multi-material adhesive joints. *Composites, Part B* **2015**, *79*, 621−630.

(7) Schindler, M.; Koller, M.; Müller-Buschbaum, P. Pressure-sensitive adhesives under the influence of relative humidity: Inner structure and failure mechanisms. *ACS Appl. Mater. Interfaces* **2015**, *7*, 12319−12327.

(8) Tan, K. T.; Vogt, B. D.; White, C. C.; Steffens, K. L.; Goldman, J.; Satija, S. K.; Clerici, C.; Hunston, D. L. On the origins of sudden adhesion loss at a critical relative humidity: Examination of bulk and interfacial contributions. *Langmuir* **2008**, *24*, 9189−9193.

(9) Waite, J. H. Nature's underwater adhesive specialist. *Int. J. Adhes. Adhes.* **1987**, *7*, 9−14.

(10) Hennebert, E.; Maldonado, B.; Ladurner, P.; Flammang, P.; Flammang, P. Experimental strategies for the identification and characterization of adhesive proteins in animals : a review. *Interface Focus* **2015**, *5*, 20140064.

(11) Amarpuri, G.; Zhang, C.; Diaz, C.; Opell, B. D.; Blackledge, T. A.; Dhinojwala, A. Spiders tune glue viscosity to maximize adhesion. *ACS Nano* **2015**, *9*, 11472−11478.

(12) Lin, H.; Lizarraga, L.; Bottomley, L. A.; Carson Meredith, J. Effect of water absorption on pollen adhesion. *J. Colloid Interface Sci.* **2015**, *442*, 133−139.

(13) Niewiarowski, P. H.; Lopez, S.; Ge, L.; Hagan, E.; Dhinojwala, A. Sticky gecko feet: the role of temperature and humidity. *PLoS One* **2008**, *3*, e2192.

(14) Opell, B. D.; Karinshak, S. E.; Sigler, M. A. Environmental response and adaptation of glycoprotein glue within the droplets of viscous prey capture threads from araneoid spider orb-webs. *J. Exp. Biol.* **2013**, *216*, 3023−3034.

(15) Stellwagen, S. D.; Opell, B. D.; Short, K. G. Temperature mediates the effect of humidity on the viscoelasticity of glycoprotein glue within the droplets of an orb-weaving spider's prey capture threads. *J. Exp. Biol.* **2014**, *217*, 1563−1569.

(16) Opell, B. D.; Karinshak, S. E.; Sigler, M. A. Humidity affects the extensibility of an orb-weaving spider's viscous thread droplets. *J. Exp. Biol.* **2011**, *214*, 2988−2993.

(17) Sahni, V.; Dhinojwala, A.; Opell, B. D.; Blackledge, T. A. In *Biotechnology of Silk*; Asakura, T., Miller, T., Eds.; Springer: Dordrecht, Netherlands, 2014; Vol. 5, pp 203−217.

(18) Sahni, V.; Blackledge, T. A.; Dhinojwala, A. A review on spider silk adhesion. *J. Adhes.* **2011**, *87*, 595−614.

(19) Opell, B. D.; Jain, D.; Dhinojwala, A.; Blackledge, T. A. Tuning orb spider glycoprotein glue performance to habitat humidity. *J. Exp. Biol.* **2018**, *221*, 161539.

(20) Blackledge, T. A.; Scharff, N.; Coddington, J. A.; Szüts, T.; Wenzel, J. W.; Hayashi, C. Y.; Agnarsson, I. Reconstructing web evolution and spider diversification in the molecular era. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 5229−5234.

(21) Opell, B. D.; Hendricks, M. L. The adhesive delivery system of viscous capture threads spun by orb-weaving spiders. *J. Exp. Biol.* **2009**, *212*, 3026−3034.

(22) Opell, B. D.; Hendricks, M. L. Adhesive recruitment by the viscous capture threads of araneoid orb-weaving spiders. *J. Exp. Biol.* **2007**, *210*, 553−560.

(23) Townley, M. A.; Pu, Q.; Zercher, C. K.; Neefus, C. D.; Tillinghast, E. K. Small organic solutes in sticky droplets from orb webs of the spider *Zygiella atrica* (Araneae; Araneidae):*β*-alaninamide is a novel and abundant component. *Chem. Biodiversity* **2012**, *9*, 2159−2174.

(24) Townley, M. A.; Tillinghast, E. K.; Neefus, C. D. Changes in composition of spider orb web sticky droplets with starvation and web removal, and synthesis of sticky droplet compounds. *J. Exp. Biol.* **2006**, *209*, 1463−1486.

(25) Townley, M. A.; Bernstein, D. T.; Gallagher, K. S.; Tillinghast, E. K. Comparative study of orb web hygroscopicity and adhesive spiral composition in three araneid spiders. *J. Exp. Zool.* **1991**, *259*, 154−165.

(26) Sinohara, H.; Tillinghast, E. K. Carbohydrates associated with the orb web protein of *Argiope Aurantia*. *Biochem. Int.* **1984**, *9*, 315−317.

(27) Tillinghast, E. K. Selective removal of glycoproteins from the adhesive spiral of the spiders orb web. *Naturwissenschaften* **1981**, *68*, 526−527.

(28) Anderson, C. M.; Tillinghast, E. K. GABA and taurine derivatives on the adhesive spiral of the orb web of *Argiope* spiders, and their possible behavioural significance. *Physiol. Entomol.* **1980**, *5*, 101−106.

(29) Tillinghast, E.; Huxtable, R.; Watson, W., III; Townley, M. Evidence of the presence of GABamide on the web of orb weaving spiders. *Comp. Biochem. Physiol. Part B Comp. Biochem.* **1987**, *88*, 457−460.

(30) Vollrath, F. Glycoprotein glue beneath a spider web's aqueous coat. *Naturwissenschaften* **1991**, *78*, 557−559.

(31) Vollrath, F.; Fairbrother, W. J.; Williams, R. J. P.; Tillinghast, E. K.; Bernstein, D. T.; Gallagher, K. S.; Townley, M. A. Compounds in the droplets of the orb spider's viscid spiral. *Nature* **1990**, *345*, 526−528.

(32) Sahni, V.; Miyoshi, T.; Chen, K.; Jain, D.; Blamires, S. J.; Blackledge, T. A.; Dhinojwala, A. Direct solvation of glycoproteins by salts in spider silk glues enhances adhesion and helps to explain the evolution of modern spider orb webs. *Biomacromolecules* **2014**, *15*, 1225−1232.

(33) Sahni, V.; Blackledge, T. A.; Dhinojwala, A. Viscoelastic solids explain spider web stickiness. *Nat. Commun.* **2010**, *1*, 19.

(34) Sahni, V.; Blackledge, T. A.; Dhinojwala, A. Changes in the adhesive properties of spider aggregate glue during the evolution of cobwebs. *Sci. Rep.* **2011**, *1*, 41.

(35) Amarpuri, G.; Chaurasia, V.; Jain, D.; Blackledge, T. A.; Dhinojwala, A. Ubiquitous distribution of salts and proteins in spider glue enhances spider silk adhesion. *Sci. Rep.* **2015**, *5*, 9030.

(36) Choresh, O.; Bayarmagnai, B.; Lewis, R. V. Spider web glue: two proteins expressed from opposite strands of the same DNA sequence. *Biomacromolecules* **2009**, *10*, 2852−2856.

(37) Jain, D.; Zhang, C.; Cool, L. R.; Blackledge, T. A.; Wesdemiotis, C.; Miyoshi, T.; Dhinojwala, A. Composition and function of spider glues maintained during the evolution of cobwebs. *Biomacromolecules* **2015**, *16*, 3373−3380.

(38) Townley, M. A.; Tillinghast, E. K. In *Spider Ecophysiology*; Nentwig, W., Ed.; Springer: Berlin, Heidelberg, 2013; pp 283−302.

(39) Jain, D.; Blackledge, T. A.; Miyoshi, T.; Dhinojwala, A. In *Biological Adhesives*, 2nd ed.; Smith, A., Ed.; Springer International Publishing, 2016; pp 303−319.

(40) Yancey, P. H. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* **2005**, *208*, 2819−2830.

(41) Arakawa, T.; Timasheff, S. N. Preferential interactions of proteins with salts in concentrated solutions. *Biochemistry* **1982**, *21*, 6545−6552.

(42) Opell, B. D.; Schwend, H. S. The effect of insect surface features on the adhesion of viscous capture threads spun by orb-weaving spiders. *J. Exp. Biol.* **2007**, *210*, 2352−2360.

(43) Garb, J. E.; González, A.; Gillespie, R. G. The black widow spider genus *Latrodectus* (Araneae: Theridiidae): Phylogeny, bio-

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057

geography, and invasion history. *Mol. Phylogenet. Evol.* **2004**, *31*, 1127−1142.
(44) Liao, C.; Blamires, S. J.; Hendricks, M. L.; Opell, B. D. A re-evaluation of the formula to estimate the volume of orb web glue droplets. *J. Arachnol.* **2015**, *43*, 97−100.
(45) Teraoka, M. Uber die Methylierung und Acylierung des Taurins. *Hoppe-Seyler's Z. Physiol. Chem.* **1925**, *145*, 238−243.
(46) Zheng, Y.; Bai, H.; Huang, Z.; Tian, X.; Nie, F. Q.; Zhao, Y.; Zhai, J.; Jiang, L. Directional water collection on wetted spider silk. *Nature* **2010**, *463*, 640−643.
(47) Collin, M. A.; Clarke, T. H.; Ayoub, N. A.; Hayashi, C. Y. Evidence from multiple species that spider silk glue component ASG2 is a spidroin. *Sci. Rep.* **2016**, *6*, 21589.
(48) Opell, B. D.; Hendricks, M. L. The role of granules within viscous capture threads of orb-weaving spiders. *J. Exp. Biol.* **2010**, *213*, 339−346.
(49) Gent, A. N. Adhesion and strength of viscoelastic solids. Is there a relationship between adhesion and bulk properties? *Langmuir* **1996**, *12*, 4492−4496.
(50) Burkett, J. R.; Hight, L. M.; Kenny, P.; Wilker, J. J. Oysters produce an organic− inorganic adhesive for intertidal reef construction. *J. Am. Chem. Soc.* **2010**, *132*, 12531−12533.
(51) Greene, G. W.; Thapa, R.; Holt, S. A.; Wang, X.; Garvey, C. J.; Tabor, R. F. Structure and property changes in self-assembled lubricin layers induced by calcium ion interactions. *Langmuir* **2017**, *33*, 2559−2570.
(52) Horkay, F.; Tasaki, I.; Basser, P. Osmotic swelling of polyacrylate hydrogels in physiological salt solutions. *Biomacromolecules* **2000**, *1*, 84−90.
(53) Du, J.; Zhang, X. Role of polymer−salt−solvent interactions in the electrospinning of polyacrylonitrile/iron acetylacetonate. *J. Appl. Polym. Sci.* **2008**, *109*, 2935−2941.
(54) Zhang, Z.; Moxey, M.; Alswieleh, A.; Morse, A. J.; Lewis, A. L.; Geoghegan, M.; Leggett, G. J. Effect of salt on phosphorylcholine-based zwitterionic polymer brushes. *Langmuir* **2016**, *32*, 5048−5057.
(55) Phadke, M. A.; Musale, D. A.; Kulkarni, S. S.; Karode, S. K. Poly(acrylonitrile) ultrafiltration membranes. I. Polymer-salt-solvent interactions. *J. Polym. Sci., Part B: Polym. Phys.* **2005**, *43*, 2061−2073.
(56) Tan, K.; White, C.; Hunston, D.; Gorham, J. M.; Imburgia, M. J.; Forster, A. M.; Vogt, B. D. Role of salt on adhesion of an epoxy/aluminum (oxide) interface in aqueous environments. *Polym. Eng. Sci.* **2016**, *56*, 18−26.

DOI: 10.1021/acs.biomac.8b00602
Biomacromolecules 2018, 19, 3048−3057